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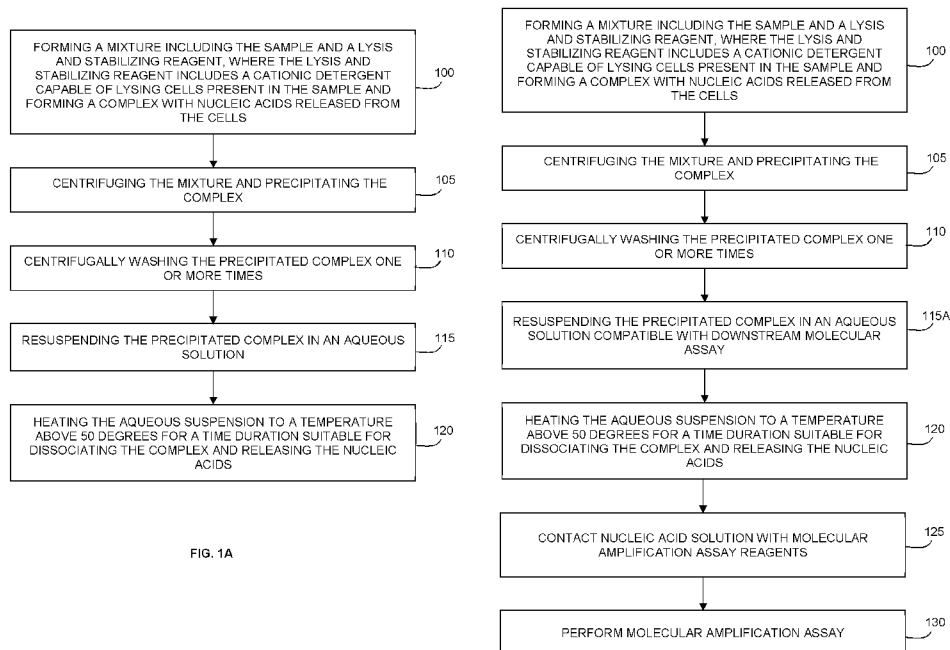
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(54) Title: METHODS OF PERFORMING NUCLEIC ACID STABILIZATION AND SEPARATION

FORMING A MIXTURE INCLUDING THE SAMPLE AND A LYSIS AND STABILIZING REAGENT, WHERE THE LYSIS AND STABILIZING REAGENT INCLUDES A CATIONIC DETERGENT CAPABLE OF LYSING CELLS PRESENT IN THE SAMPLE AND FORMING A COMPLEX WITH NUCLEIC ACIDS RELEASED FROM THE CELLS — 100

CENTRIFUGING THE MIXTURE AND PRECIPITATING THE COMPLEX — 105

CENTRIFUGALLY WASHING THE PRECIPITATED COMPLEX ONE OR MORE TIMES — 110

RESUSPENDING THE PRECIPITATED COMPLEX IN AN AQUEOUS SOLUTION — 115

HEATING THE AQUEOUS SUSPENSION TO A TEMPERATURE ABOVE 50 DEGREES FOR A TIME DURATION SUITABLE FOR DISSOCIATING THE COMPLEX AND RELEASING THE NUCLEIC ACIDS — 120

FIG. 1A

FORMING A MIXTURE INCLUDING THE SAMPLE AND A LYSIS AND STABILIZING REAGENT, WHERE THE LYSIS AND STABILIZING REAGENT INCLUDES A CATIONIC DETERGENT CAPABLE OF LYSING CELLS PRESENT IN THE SAMPLE AND FORMING A COMPLEX WITH NUCLEIC ACIDS RELEASED FROM THE CELLS — 100

CENTRIFUGING THE MIXTURE AND PRECIPITATING THE COMPLEX — 105

CENTRIFUGALLY WASHING THE PRECIPITATED COMPLEX ONE OR MORE TIMES — 110

RESUSPENDING THE PRECIPITATED COMPLEX IN AN AQUEOUS SOLUTION COMPATIBLE WITH DOWNSTREAM MOLECULAR ASSAY — 115A

HEATING THE AQUEOUS SUSPENSION TO A TEMPERATURE ABOVE 50 DEGREES FOR A TIME DURATION SUITABLE FOR DISSOCIATING THE COMPLEX AND RELEASING THE NUCLEIC ACIDS — 120

CONTACT NUCLEIC ACID SOLUTION WITH MOLECULAR AMPLIFICATION ASSAY REAGENTS — 125

PERFORM MOLECULAR AMPLIFICATION ASSAY — 130

FIG. 1B

(57) Abstract: Methods are provided for the stabilization and separation of nucleic acids from a sample via contact of the sample with a lysis and stabilization reagent that includes a cationic detergent. The cationic detergent lyses cells in the sample and stabilizes the released nucleic acids via the formation of nucleic acid – surfactant (NAS) complexes. The NAS complexes are centrifugally precipitated, washed, the resuspended in an aqueous resuspension liquid, forming a NAS complex suspension. The suspension is thermally processed to disintegrate the NAS complexes, thereby releasing the nucleic acids and forming a nucleic acid solution. In some example embodiments, the aqueous resuspension liquid is selected to be suitable for performing molecular amplification assays, such that the nucleic acid solution may be employed for performing a molecular amplification assay in the absence of further nucleic acid extraction. Examples are provided whereby the present methods are adapted for performing transcriptomic biomarker assays.

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METHODS OF PERFORMING NUCLEIC ACID STABILIZATION AND SEPARATION

CROSS-REFERENCE TO RELATED APPLICATION

5   This application claims priority to U.S. Provisional Application No. 62/419,340, titled "METHODS OF PERFORMING NUCLEIC ACID STABILIZATION AND SEPARATION" and filed on November 8, 2016, the entire contents of which is incorporated herein by reference.

10   **BACKGROUND**

        The present disclosure relates to methods of separating nucleic acids from samples. In some aspects, the present disclosure relates to methods of rapid transcriptomic profiling, including performing rapid mRNA biomarker assays on whole blood samples.

15   Cationic detergents (surfactants) have been known to be suitable as agents for the lysis of cells in a sample, and also for the stabilization of nucleic acids such as various forms of RNA. For example, in US Patents No. 5,010,183 and 5,728,822, Macfarlane teaches methods whereby cationic detergents, when mixed with a sample, effectively lyse cells within the

20   sample, while forming a complex with nucleic acids released from the cells that stabilizes the nucleic acids and prevents gene induction. Unfortunately, the subsequent processing of such complexes for the release of nucleic acids, as taught in the art, requires complicated steps that involve nucleic acid extraction, which are not readily implemented in an automated system.

25

SUMMARY

Methods are provided for the stabilization and separation of nucleic acids from a sample via contact of the sample with a lysis and stabilization reagent that includes a cationic detergent. The cationic detergent lyses cells in the sample and stabilizes the released nucleic acids via the formation of nucleic acid – surfactant (NAS) complexes. The NAS complexes are centrifugally precipitated, washed, the resuspended in an aqueous resuspension liquid, forming a NAS complex suspension. The suspension is thermally processed to disintegrate the NAS complexes, thereby releasing the nucleic acids and forming a nucleic acid solution. In some example embodiments, the aqueous resuspension liquid is selected to be suitable for performing molecular amplification assays, such that the nucleic acid solution may be employed for performing a molecular amplification assay in the absence of further nucleic acid extraction. Examples are provided whereby the present methods are adapted for performing transcriptomic biomarker assays.

Accordingly, in a first aspect, there is provided a method of processing a sample to release nucleic acids therefrom, the method comprising:

forming a mixture comprising the sample and a lysis and stabilizing reagent, the lysis and stabilizing reagent comprising a cationic detergent, the cationic detergent being capable of lysing cells present in the sample and forming a complex with nucleic acids released from the cells, wherein the lysis and stabilizing reagent is provided in an amount sufficient to stabilize and prevent nucleic acid degradation;

separating the complex from the mixture;

resuspending the separated complex in an aqueous solution, thereby obtaining an aqueous suspension comprising the separated complex; and

heating the aqueous suspension to a temperature above 50 degrees Celsius for a time duration suitable for dissociating the complex, thereby releasing the nucleic acids therefrom.

In another aspect, there is provided a method of a performing a molecular assay on a sample to detect nucleic acids released therefrom, the method comprising:

a) forming a mixture comprising the sample and a lysis and stabilizing reagent, the lysis and stabilizing reagent comprising a cationic detergent, the cationic detergent being capable of lysing cells present in the sample and forming a complex with nucleic acids released from the cells, wherein the lysis and stabilizing reagent is provided in an amount sufficient to stabilize and prevent nucleic acid degradation;

b) separating the complex from the mixture;

c) resuspending the separated complex in an aqueous solution, thereby obtaining an aqueous suspension comprising the separated complex;

d) heating the aqueous suspension to a temperature above 50 degrees Celsius for a time duration suitable for dissociating the complex, thereby forming a nucleic acid solution comprising the nucleic acids released from the complex; and

e) performing an assay to detect nucleic acids present in the nucleic acid solution, wherein the assay is performed in absence of nucleic acid extraction.

A further understanding of the functional and advantageous aspects of

the disclosure can be realized by reference to the following detailed

description and drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

5   Embodiments will now be described, by way of example only, with

reference to the drawings, in which:

FIG. 1A is a flow chart illustrating an example method of processing a

sample to separate nucleic acids using a cationic detergent.

FIG. 1B is a flow chart illustrating an example method of processing a

10   sample to separate nucleic acids using a cationic detergent, and subsequently

performing a molecular assay in the absence of a nucleic acid extraction step.

FIG. 2A is a table showing processing parameters pertaining to the

results shown in FIGS. 2B-2E.

FIG. 2B plots the real time reverse transcription PCR (real-time RT-

15   PCR) and real-time PCR signals of lysosomal associated membrane protein 1

(LAMP1) mRNA and genomic DNA detection obtained by processing a whole

blood sample.

FIG. 2C plots the real time reverse transcription PCR (real-time RT-

PCR) and real-time PCR signals of Glyceraldehyde 3-phosphate

20   dehydrogenase (GAPDH) mRNA and genomic DNA detection obtained by

processing a whole blood sample.

FIG. 2D plots the melting peaks of the amplicons at the end of the real-

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time RT-PCR and real-time PCR of FIG. 2A.

FIG. 2E plots the melting peaks of the amplicons at the end of the real-time RT-PCR and real-time PCR of FIG. 2B.

FIG. 3 is a table showing processing parameters pertaining to the results shown in FIG. 4.

FIG. 4 is a table showing biomarker and housekeeping gene assay results over a range of different storage conditions and times.

FIG. 5 is a table showing processing parameters pertaining to the results shown in FIG. 6.

FIG. 6 is a table showing biomarker and housekeeping gene assay results over a range of different storage conditions and times.

FIG. 7 is a table showing processing parameters pertaining to the results shown in FIG. 8A-8B.

FIG. 8A is a table showing the dependence of nucleic acid-surfactant complex disintegration on temperature as evidenced by the real-time RT-PCR assay cycle number.

FIG. 8B plots the dependence of nucleic acid - surfactant complex (NAS-complex) disintegration on the processing temperature as evidenced by the real-time RT-PCR assay cycle number.

FIG. 9 is a table showing processing parameters pertaining to the results shown in FIG. 10.

FIG. 10 is a table showing the dependence of the real-time RT-PCR assay cycle number on thermal incubation time at a temperature of 95°C.

FIG. 11 is a table showing processing parameters pertaining to the results shown in FIG. 12.

FIG. 12 is a table showing the effect of different heat treatment methods on RT-PCR assay cycle number.

FIG. 13 is a table showing processing parameters pertaining to the results shown in FIG. 14.

5   FIG. 14 is a table showing the effect of wash cycles on RT-PCR assay cycle number.

DETAILED DESCRIPTION

Various embodiments and aspects of the disclosure will be described

10   with reference to details discussed below. The following description and drawings are illustrative of the disclosure and are not to be construed as limiting the disclosure. Numerous specific details are described to provide a thorough understanding of various embodiments of the present disclosure. However, in certain instances, well-known or conventional details are not

15   described in order to provide a concise discussion of embodiments of the present disclosure.

As used herein, the terms "comprises" and "comprising" are to be construed as being inclusive and open ended, and not exclusive. Specifically, when used in the specification and claims, the terms "comprises" and

20   "comprising" and variations thereof mean the specified features, steps or components are included. These terms are not to be interpreted to exclude the presence of other features, steps or components.

As used herein, the term "exemplary" means "serving as an example, instance, or illustration," and should not be construed as preferred or

25   advantageous over other configurations disclosed herein.

As used herein, the terms "about" and "approximately" are meant to cover variations that may exist in the upper and lower limits of the ranges of values, such as variations in properties, parameters, and dimensions. Unless otherwise specified, the terms "about" and "approximately" mean plus or

5   minus 25 percent or less.

It is to be understood that unless otherwise specified, any specified range or group is as a shorthand way of referring to each and every member of a range or group individually, as well as each and every possible sub-range or sub -group encompassed therein and similarly with respect to any sub-

10   ranges or sub-groups therein. Unless otherwise specified, the present disclosure relates to and explicitly incorporates each and every specific member and combination of sub-ranges or sub-groups.

As used herein, the term "on the order of", when used in conjunction with a quantity or parameter, refers to a range spanning approximately one

15   tenth to ten times the stated quantity or parameter.

As used herein, the phrase "nucleic acid" refers to a single stranded or double stranded nucleic acid sequence that may consist of deoxyribonucleotides (DNA) or ribonucleotides (RNA), RNA/DNA hybrids or may be amplified cDNAor amplified genomic DNA, or a combination thereof.

20   Various example embodiments of the present disclosure relate to the use of cationic detergents during sample preparation. Cationic detergents (surfactants) have been known to be suitable as agents for the lysis of cells in a sample, and also for the stabilization of nucleic acids such as DNA and various forms of RNA (e.g. mRNA, tRNA, snRNA, lower molecular weight

25   (LMW)RNA, rRNA and cRNA). For example, in US Patents No. 5,010,183 and

5,728,822, which are incorporated herein by reference in their entirety, Macfarlane teaches methods whereby cationic detergents, when mixed with a sample, effectively lyse cells within the sample, while forming a complex with nucleic acids released from the cells that stabilizes the nucleic acids and

5   prevents gene induction.

Referring now to FIG. 1A, a flow chart is shown that illustrates an example implementation of stabilizing and separating nucleic acids from a sample. In step 100 of FIG. 1, a sample, such as a whole blood sample, is combined with a lysis and stabilization reagent that includes a cationic

10   detergent (cationic surfactant) to form a mixture. The cationic detergent lyses cells within the sample and stabilizes the nucleic acids, forming a nucleic acid – surfactant (NAS) complex, as described above.

The mixture is then centrifuged in a centrifugation vessel such that the NAS complex is precipitated, as shown at step 105. The centrifugation is

15   performed at a speed and time duration that are suitable for the pelleting of the precipitate. Non-limiting examples of centrifugation parameters are 100g to 15,000g for a time duration ranging from 1 to 20 minutes. After removal of the supernatant formed above the precipitate, the precipitate pellet is washed according to one or more wash cycles 110, each wash cycle involving the

20   addition of a volume of an appropriate aqueous liquid to the centrifugation vessel, centrifuging the centrifugation vessel for a suitable time to re-pellet the complex, and removing the supernatant (or a substantial portion thereof). It has been found that a phosphate buffer (PB) having an ionic strength of 0.1 mM to 10 mM and pH 7.4, provides a suitable wash liquid, but it will be

25   understood that other aqueous wash solutions may be used in the alternative,

such as RNase free water.

Unlike the teachings in the art, such as those of Macfarlane, the present inventors have found that the nucleic acids bound to the NAS complex can be liberated, in a form that is suitable for downstream molecular

5   assays, using a thermal processing step that causes disintegration (e.g. dissociation, disruption) of the complex.

Prior to the thermal disintegration step, the washed pellet is resuspended in an aqueous liquid. The aqueous liquid may be the same liquid as the aqueous wash liquid, or may be a different aqueous liquid. In one

10   example implementation, the aqueous liquid is a phosphate buffer as described in the Examples below.

Having resuspended the NAS-complex in the aqueous liquid, the resulting suspension can be thermally processed to disintegrate the NAS complex and form a nucleic acid solution. This thermal disintegration step is

15   shown at 120 in FIG. 1A. In one example embodiment, thermal disintegration may be performed by heating the NAS complex suspension above a pre-selected threshold temperature associated with disintegration, and maintaining the temperature of the suspension above the threshold temperature for a prescribed time duration. It will be understood that the

20   thermal processing parameters for achieving NAS complex disintegration may vary depending on the type of cationic detergent that is employed.

In one example embodiment, the nucleic acid solution may be subjected to one or more centrifugation or filtration steps. For example, the nucleic acid solution may be centrifuged and the supernatant may be

25   retained. Additionally or alternatively, the nucleic acid solution may be passed

through a filter, and the filtrate may be collected.

The skilled artisan will be able to select a suitable temperature and time duration by performing a series of experiments at different thermal processing temperatures and thermal processing times, and measuring, directly or

5   indirectly, the effect of the thermal processing conditions on the disintegration of the NAS complex. An indirect assessment of disintegration can be made, for example, by monitoring the quantity of nucleic acids recovered and amplified via a real-time reverse transcription assay, as described in the Examples below. A direct assessment of the disintegration of the NAS

10   complex can be made, for example, by a detection modality that is sensitive to the presence of residual complexes that are not disintegrated, e.g. via optical absorbance or light scattering measurements.

For example, as shown below in the Examples, it has been found that for the case of samples processed via PAXgene™ tubes, disintegration of the

15   complex occurs above a temperature of 50°C when a thermal incubation time of 10 minutes is selected. However, further disintegration appears to occur as the temperature is increased, such that, for example, thermal processing temperatures exceeding 55°C, 60°C, 65°C, 70°C, 75°C, 80°C may be preferred.

20   It will be understood that the thermal processing step described above may be implemented according to a wide variety of example implementations. In one example implementation, the resuspended NAS complex suspension may be disrupted by subjecting the complex to Joule heating via the generation of an internal electric current within the suspension.

25   For example, the rapid Joule heating method ("flash heating") disclosed

in US Patent Application No. 2014/0004501, which is incorporated herein by reference in its entirety, may be employed to achieve thermal processing for achieving NAS-complex disintegration. This rapid Joule heating is performed by flowing the NAS-complex suspension into an electrical processing

5   chamber having electrodes for the application of an electric field thereacross. The electrodes may be subjected to AC electrical voltage, such that the suspension is internally heated via Joule heating.

In one example embodiment, one or more of the electrodes may be blocking electrodes to prevent the formation of a Faradaic current in the

10   suspension. The electrodes may be provided as a thin conductive substrate and with a thin dielectric coating in contact with the suspension within the electrical processing chamber, where the surface profile of the conductor and dielectric is microstructured for surface area enhancement such that the blocking electrode surface area substantially exceeds the surface area of the

15   corresponding flat surface. The large capacitance thereby achieved enables a charging time greater than one microsecond, such as on the order of tens of microseconds. The capacitance of the blocking layer can also be enhanced by providing a thin dielectric layer having a high dielectric constant. In one example implementation, the metal substrate is aluminum, and the dielectric

20   layer is aluminum oxide (Al_2O_3). This aluminum oxide (Al_2O_3) dielectric layer is formed by electrochemically oxidizing the aluminum (anodized aluminum). In order to increase the effective surface by as much as 100 times and to provide a corresponding increase to the capacitance per unit nominal area, the electrode is etched with a dense network of microscopic cavities and

25   tunnels.

According to the present example implementation, the voltage may be applied such that the temperature of the suspension rises to, and optionally exceeds, 100°C within a timescale of less than one second. The suspension may be superheated during thermal processing by regulating an internal
5   pressure within the chamber.

According to the present example implementation, the aqueous resuspension liquid into which the NAS-complex is resuspended following the final washing step is selected to have a suitable ionic strength for generating Joule heating via the generation of ionic currents, and the electric field is
10   provided with a sufficient frequency to support internal Joule currents and avoid substantial electric field screening effects. For example, the ionic strength of the aqueous resuspension liquid may be selected to be below a maximal value in order to support the establishment of an effective electric field with a suitable timescale for effecting electrical processing. The specific
15   maximal value or range of suitable values of the ionic strength will mainly depend on the capability of the applied voltage source to deliver high voltage along with the corresponding current over the timescale over which the processing is desired to occur. It is to be understood that those skilled in the art may perform routine experimentation in order to determine a suitable
20   upper limit or range of values for the ionic strength in a given application. According to other example implementations, the ionic strength of the aqueous resuspension liquid may range from approximately 0.1 mM < I < 100 mM.

Without intending to be limited theory, it is presently contemplated that
25   the disintegration efficiency of the present rapid Joule heating method may be

dependent on the heating rate in order to effect rapid disintegration on thermal

processing timescales of less than one second. Accordingly, in selected

example embodiments, the heating rate of the suspension for the electrical

processing may be greater than approximately 250°C/s, or greater than

5   approximately 2000°C/s. Furthermore, without intending to be limited theory, it

is presently contemplated that the disintegration efficiency of the present rapid

Joule heating method may depend on the electric field that is applied within

the suspension liquid during thermal processing in order to effect rapid

disintegration on thermal processing timescales of less than one second.

10   Accordingly, in selected example embodiments, voltages may be applied to

the sample in order achieve internal electric fields ranging from 200 V/cm to

50 kV/cm, or 200 V/cm to 2 kV/cm, or 200 V/cm to 30 kV/cm, or 2 kV/cm to 50

kV/cm, or 2 kV/cm to 30 kV/cm.

         As described above, the lysis and stabilizing reagent employed

15   according to various example methods of the present disclosure includes a

cationic detergent. The cationic reagent may be selected to be suitable for the

lysis of a wide range of cells, including, but not limited to, erythrocytes,

leukocytes, and microbial cells. In some example implementations, the lysis

and stabilizing agent may be suitable for the lysis of viruses and the

20   stabilization of viral nucleic acids. The nucleic acids may be present extra-

and/or intracellularly in the biological sample.

         It will be understood that the prior art teaches a wide range of suitable

cationic detergent reagent formulations for effecting lysis and stabilization. In

some example embodiments, the lysis and stabilizing reagent may include

25   one or more cationic detergents, such as, not limited to, quaternary amine

surfactants. Examples of suitable cationic reagents are disclosed by

Macfarlane in US Patent Nos. 5,010,183 and 5,728,822 and by Augello et al.

in US Patent No. 6,602,718.

The cationic detergent may be provided within a collection vessel, such

5   as an evacuated collection tube, such as those disclosed by Augello et al. in

US Patent No. 6,602,718, and such as the commercially available PAXgene™

collection tube (which contains <10% by weight of

Tetradecyltrimethylammonium oxalate in an acidic solution (pH 3.7) of 2-4%

Tartaric acid).

10   In some example embodiments, the lysis and stabilizing reagent may

include one or more additional components, in addition to the cationic

detergent. Nonlimiting examples of additional components include additional

detergents, chaotropic salts, ribonuclease inhibitors, chelating agents and

mixtures thereof.

15   It will be understood that although many of the example

implementations described herein involve the processing of whole blood

samples, it will be understood that the present disclosure is not intended to be

limited to whole blood processing. For example, in some example

implementations, the sample may be a blood containing sample that includes

20   one or more types of blood cells. Non-limiting examples of biological samples

other than whole blood include cell-containing compositions such as red blood

cell concentrates, platelet concentrates, leukocyte concentrates, plasma,

serum, urine, bone marrow aspirates, cerebral spinal fluid, tissue, cells, and

other body fluids. The samples may be from human or animal origin, and the

25   sample can be solid or liquid in nature (or a combination thereof). Solid

samples may be processed by known pre-analytic methods, such as dissolving the solids in a suitable solution.

Although the preceding example method is described as employing centrifugation for the separation and washing of the NAS complex, it will be

5   understood that the NAS complex may alternatively be separated via filtration.

Referring now to FIG. 1B, a flow chart is provided that illustrates an example implementation of a method of performing a molecular assay after thermal processing of the suspension and disintegration of the NAS complex, in the absence of an additional nucleic acid extraction step. As in FIG. 1A, the

10   sample is treated by exposure to a lysis and stabilizing reagent, and the resulting NAS complexes are washed according to the centrifugal processing method described above. However, as shown in step 115A, the aqueous resuspension liquid (for resuspension of the NAS complex prior to thermal disintegration) is selected to be compatible with molecular amplification

15   assays, such as, but not limited to, PCR, real-time PCR, reverse transcription PCR (RT-PCR) and real-time RT-PCT, such that when the nucleic acids are liberated via thermal disintegration, the resulting nucleic acid solution can be mixed with molecular amplification reagents without requiring an intermediate nucleic acid extraction step. This capability of the present method to perform

20   nucleic acid separation in the absence of an intermediate nucleic acid extraction step may be beneficial when implementing the present methods on an automated platform.

After having dissociated the NAS complexes in step 120, the nucleic acid solution is contacted with assay reagents at step 125 for performing a

25   molecular amplification assay. The assay is subsequently performed in step

130.

In one example embodiment, the nucleic acid solution may be subjected to one or more centrifugation or filtration steps prior to performing the assay. For example, the nucleic acid solution may be centrifuged and the

5   supernatant may be retained. Additionally or alternatively, the nucleic acid solution may be passed through a filter, and the filtrate may be collected.

In some example implementations, the example method illustrated in FIG. 1B may be adapted to performing one or more transcriptomic biomarker assays. For example, the example method may be adapted to assess a

10   partial transcriptomic profile of leukocytes based on a blood sample drawn from a subject. The example method may be employed to infer the state of subject's immune response to an infection, if present, such as a microbial infection.

In one illustrative example method, two gene sets are employed for

15   determining a transcriptomic host response: an infection state indicator gene set (ISIG) comprising one or more transcriptomic biomarker genes, and a housekeeping gene set (HKG) comprising one or more nucleic acid housekeeping gene targets. The expression profile of HKG set is selected to not be significantly influenced by the health state of the host, while the

20   expression profile of the ISIG set significantly changes if the host is infected by microbial cells.

In order to assess the host's state of health a volume of blood is drawn into an appropriate blood collection tube containing a cationic detergent. The cationic detergent lyses leukocytes in the sample and stabilizes mRNA from

25   the leukocytes in the time interval between blood draw and the start of sample

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processing. Steps 100-120 of FIG. 1B are performed, yielding a nucleic acid solution ready for PCR and/or RT-PCR analysis. The resulting nucleic acid solution is assayed in spatially multiplexed real-time RT-PCR array and the relative level of each target mRNA from ISIG set is determined relative to its

5   genomic DNA or relative to the level of at least one mRNA belonging to HKG set.

As described above, the nucleic acid solution obtained by performing step 120 is ready for proceeding with reverse transcription (RT) and polymerase chain reaction (PCR) assays for determining the average level of

10   target mRNA in the original leukocytes. It will be understood that other assays that PCR may be employed to detect and/or quantify the mRNA, such as sequencing or other nucleic acid amplification assays.

In one example embodiment, the nucleic acid solution is split and is delivered into multiple reaction chambers and is mixed with reagents provided

15   for performing reverse transcription and PCR. In one example embodiment, each chamber is provided with specific primers targeting the desired mRNA strand. Thus, in step 130, target mRNAs are converted to cDNA through the enzymatic reaction. During the subsequent thermal cycling, the cDNA and its corresponding DNA segment on the genomic DNA are amplified to amplicons.

20   In one example embodiment, the forward and reverse primers are designed such that the target section on the genomic DNA includes an intron segment. Thus, the amplicons originating from the mRNA and the genomic DNA will have different lengths and can be differentiated in step 130. In such a case, amplified mRNA and amplified genomic DNA can be separately

25   identified, for example, by subjecting the amplicons to melting curve analysis.

In an alternative example embodiment, the reverse primer is designed to target one intron-exon junction (splice junction) region. This suppresses the conversion of pre-mRNA to cDNA during reverse transcription stage. Moreover, during PCR stage gDNA will have little contribution to the amplified

5   product. In one example implementation, each reaction vessel may be provided with two pairs of reverse and forward primers; one pair for a gene from the ISIG set and another pair for a gene from the HKG set. The length of the cDNA are selected, through primer pair design, such that the melt peak corresponding to the two types of amplicons are sufficiently separated. Then,

10   the transcriptomic level of the target gene will be determined by referencing the amplitude of its corresponding melt peak to the amplitude of the house keeping gene's melt peak.

EXAMPLES

15   The following examples are presented to enable those skilled in the art to understand and to practice embodiments of the present disclosure. They should not be considered as a limitation on the scope of the disclosure, but merely as being illustrative and representative thereof.

The examples described below illustrate non-limiting example

20   implementations of some of the methods described above. In the forthcoming examples, 2.5 milliliters of whole blood was added to a PAXgene™ tube, forming a mixture in which nucleic acids from the leukocytes were stabilized as nucleic acid – surfactant complexes (NAS-complexes), and the mixture was stored at room temperature and processed after a storage time delay, t_{st},

25   ranging from 15 minutes to 3 days. In some experiments, the mixture was

frozen temperature and was processed after the storage time delay at room temperature for 1 hour to 1 day.

For the first sedimentation step, 1 mL of the mixture was added to a 0.8 mM pH 7.4 phosphate buffer (PB) with a volume V_b ranging from 0 to 10 mL in a centrifuge tube, and centrifuged at 4000g for a first sedimentation time, t_{se}, ranging from of 1 to 10 minutes. The supernatant was then removed and 100 μL of the residual liquid was left behind with the NAS-complex pellet in the tube.

Three to four cycles of washes were then performed, with each wash cycle being performed by adding 900 μL of PB buffer to the vessel, resuspending the pellet, centrifuging for 3 minutes at 4000g, and removing 900 μL of the supernatant, such that a substantial volume of the supernatant liquid was removed. After the final wash, the NAS-complex was resuspended in the remaining approximately 100 μL of residual liquid.

The resulting suspension was subjected to a NAS-complex disintegration step by heat treatment at temperature T_d for a period of t_d minutes, thereby generating a nucleic acid solution. Both T_d and t_d were varied experimentally as shown in the examples presented below.

The resulting nucleic acid solution was passed through a filter with 0.45μm pore size to remove residual complexes. One μL of the nucleic acid solution was then added to 1 μL of specific primer set (0.25 μM) and 3 μL of the respective mastermix and was subjected to a real-time RT-PCR or PCR assay. The example primer pairs that were employed in the present examples respectively target one infection state indicator gene (belonging to ISIG), lysosomal associated membrane protein 1(LAMP1), and one housekeeping

gene set (belonging to HKG), Glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

The LAMP1 detection primers used in the following examples were forward (5'- ACCGTCCTGCTCTTCCAGTT -3') and reverse (5'-

5 GGCAGGGTCTCTGGCGTC -3'). The GAPDH detection primers used in the following examples were forward (5'- GCCACATCGCTCAGACACC -3') and reverse (5'- GTTAAAAGCAGCCCTGGTGACC -3').

The RT-PCR mastermix included Taq polymerase and reverse transcriptase enzymes in appropriate buffer and used SYBR Green dye for

10 signal detection. When performing the real-time PCR assay, the reverse transcription enzyme was not included in the mastermix. The assay signal was analyzed and two parameters were found: the threshold cycle, C_T, and the derivative melt peak (ΔT_m).

15 **EXAMPLE 1: Demonstration Transcription Profiling using Method Involving Two Selected Genes**

The present example demonstrates the ability of an implementation of the aforementioned methods to detect mRNA along with its corresponding gDNA in a single reaction well, while differentiating their contribution to the

20 amplified product by interrogating melt peaks. One mL of a sample/cationic detergent mixture from a PAXgene™ tube was processed with the parameters presented in FIG. 2A. Two amplification assays were performed in separate wells; a RT-PCR assay and a PCR only assay. The resulting amplification curves are presented in FIGS. 2B and 2C. The position of peaks in the

25 corresponding derivative melt curves of FIGS. 2D and 2E indicate that

aforementioned methods involving the use of heat to liberate nucleic acids from the NAS-complex are suitable for the detection and differentiation of mRNA and its corresponding gDNA.

5   **EXAMPLE 2: Processing of Sample/Cationic Detergent Mixture at Various Storage Time Delays and Storage Temperatures**

The present example is provided to demonstrate the robustness of the cationic detergent and sample mixture in the context of varying storage temperature and storage time. The processing parameters are presented in

10   FIG. 3. The assay results of FIG. 4 indicate that the mixture can be stored in room temperature for times durations exceeding 3 days without compromising the ability to detect mRNA level in leukocytes using the example methods disclosed herein. Moreover, the results shown in FIG. 4 demonstrate that the mixture can be frozen for various time intervals without substantially affecting

15   assay performance.

EXAMPLE 3: Processing of Sample/Cationic Detergent Mixture at Different First Sedimentation Times

The present example is provided to demonstrate that the NAS-complex

20   is readily sedimented regardless of the storage condition. The processing parameters are presented in FIG. 5 and the assay results are presented in FIG. 6.

EXAMPLE 4: Processing of Sample/Cationic Detergent Mixture Followed

25   **by Heat Treatment at Different Temperatures**

The present example is provided to investigate of the temperature, T_d, at which the NAS-complex disintegration is accomplished. The processing parameters are presented in FIG. 7. The results, which are presented in FIG. 8A, are also plotted in FIG. 8B. As it is observed in the figures, a processing temperature T_d below 80°C the results in higher real-time RT-PCR and real-time PCR cycle numbers, indicating a clear temperature dependence. Without intending to be limited by theory, it is believed that this assay result temperature dependence corresponds to a temperature dependence of disintegration (e.g. dissociation, disruption) of the NAS-complex.

EXAMPLE 5: Processing of Sample/Cationic Detergent Mixture Followed by Heat Treatment at T_d= 95 for Different Thermal Processing Times

The present example is provided to demonstrate the dependence of the mRNA detection performance of an example implementation of the preceding method on the heat induced NAS-complex disintegration time. The processing parameters are presented in FIG. 9. The results, which are presented in FIG. 10, indicate that subjecting the washed NAS-complex to sufficiently high temperature for a period over 1 minute is sufficient to accomplish mRNA detection. Without intending to be limited by theory, it is expected that even shorter thermal processing times may be achievable at higher processing temperatures, and/or in the presence of a rapid rate of increase in temperature during heating.

EXAMPLE 6: Processing of Sample/Cationic Detergent Mixture Followed by Heat Treatment or Flash-Heating Treatment

The present example demonstrates the possibility of employing rapid

Joule heating ("flash-heating") based thermal processing for achieving NAS-

complex disintegration. In the current example, the NAS-complex suspension

was introduced into an electrical processing chamber having a pair of

5   electrodes for generating internal Joule heating. The electrical processing

chamber had a volume of 20 µL and a thickness of 200 µm, with top and

bottom electrodes. The electrodes were formed from microstructured

aluminum with a conformal aluminum oxide dielectric layer, as described

above. The NAS-complex was resuspended, after the final wash step, in PB

10   buffer having an ionic strength of 0.8 mM. Electrical excitation was provided in

the form of a series of square wave AC pulses of duration 50 µs and

amplitude of 200 V, where the pulses were applied to the chamber for a

duration 50 ms. It is believed that the temperature within the chamber

exceeded 100°C and that the heating rate exceeded 1500°C/s. The

15   processing parameters are presented in FIG. 11. The assay results of FIG. 12

indicate that rapid Joule heating is similar to conventional heating in terms of

the NAS-complex disintegration efficiency, while achieving NAS-complex

disintegration on a much faster timescale.

20   **EXAMPLE 7: Dependence of RT-PCR Signal on Number of Washes**

The present example demonstrates the effect of additional centrifugal

wash cycles on the detection of an mRNA target via real-time RT-PCR. The

processing parameters are presented in FIG. 13 and the assay results are

presented in FIG. 14. The results demonstrate that in the present case, at

25   least two wash cycles were necessary to detecting mRNA target via RT-PCR

assay with a PCR cycle number of less than 30.

The specific embodiments described above have been shown by way of example, and it should be understood that these embodiments may be susceptible to various modifications and alternative forms. It should be further understood that the claims are not intended to be limited to the particular forms disclosed, but rather to cover all modifications, equivalents, and alternatives falling within the spirit and scope of this disclosure.

THEREFORE WHAT IS CLAIMED IS:

1. A method of processing a sample to release nucleic acids therefrom, the method comprising:

forming a mixture comprising the sample and a lysis and stabilizing reagent, the lysis and stabilizing reagent comprising a cationic detergent, the cationic detergent being capable of lysing cells present in the sample and forming a complex with nucleic acids released from the cells, wherein the lysis and stabilizing reagent is provided in an amount sufficient to stabilize and prevent nucleic acid degradation;

separating the complex from the mixture;

resuspending the separated complex in an aqueous solution, thereby obtaining an aqueous suspension comprising the separated complex; and

heating the aqueous suspension to a temperature above 50 degrees Celsius for a time duration suitable for dissociating the complex, thereby releasing the nucleic acids therefrom.

2. The method according to claim 1 wherein the aqueous suspension is heated above at least 70 degrees Celsius.

3. The method according to claim 1 wherein the aqueous suspension is heated above at least 80 degrees Celsius.

4. The method according to any one of claims 1 to 3 wherein the aqueous suspension is heated for at least one minute.

5. The method according to any one of claims 1 to 4 wherein the aqueous suspension is heated by Joule heating through an electric current applied thereto.

6. The method according to any one of claims 1 to 5 wherein the sample is a blood containing sample.

7. The method according to any one of claims 1 to 5 wherein the sample is a whole blood sample.

8. The method according to claim 7 wherein the whole blood sample is sampled directly into a blood collection vessel containing the lysis and stabilizing reagent.

9. The method according to claim 8 wherein the blood collection vessel is a PAXgene(TM) tube.

10. The method according to any one of claims 1 to 9 wherein the complex is separated centrifugally by:

      centrifuging the mixture and precipitating the complex, thereby obtaining a precipitated complex;

      centrifugally washing the precipitated complex one or more times.

11. The method according to any one of claims 1 to 9 wherein the complex is

separated by filtration.

12. The method according to any one of claims 7 to 9 wherein the complex is separated centrifugally by:

        centrifuging the mixture and precipitating the complex, thereby obtaining a precipitated complex;

        centrifugally washing the precipitated complex two or more times.

13. A method of a performing a molecular assay on a sample to detect nucleic acids released therefrom, the method comprising:

        a) forming a mixture comprising the sample and a lysis and stabilizing reagent, the lysis and stabilizing reagent comprising a cationic detergent, the cationic detergent being capable of lysing cells present in the sample and forming a complex with nucleic acids released from the cells, wherein the lysis and stabilizing reagent is provided in an amount sufficient to stabilize and prevent nucleic acid degradation;

        b) separating the complex from the mixture;

        c) resuspending the separated complex in an aqueous solution, thereby obtaining an aqueous suspension comprising the separated complex;

        d) heating the aqueous suspension to a temperature above 50 degrees Celsius for a time duration suitable for dissociating the complex, thereby forming a nucleic acid solution comprising the nucleic acids released from the complex; and

        e) performing an assay to detect nucleic acids present in the nucleic acid solution, wherein the assay is performed in absence of nucleic acid

extraction.

14. The method according to claim 13 wherein the assay is a biomarker

assay performed by contacting at least a portion of the nucleic acid solution

with assay reagents comprising a primer set configured to detect a biomarker.

15. The method according to claim 14 wherein the biomarker is a messenger

RNA biomarker.

16. The method according to claim 15 wherein the messenger RNA

biomarker is associated with a genomic region comprising at least a portion of

an intron.

17. The method according to claim 14 wherein the biomarker is associated

with the response of a host to an infection.

18. The method according to claim 17 wherein the assay reagent are first

assay reagents, and the primer set is a first primer set, the method further

comprising:

 performing a housekeeping gene assay by contacting at least a

portion of the nucleic acid solution with second assay reagents comprising a

second primer set configured to detect housekeeping messenger RNA

associated with a housekeeping gene;

 wherein the biomarker assay generates a biomarker reverse

transcription assay result quantifying an amount of the biomarker present in

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the nucleic acid solution, and the housekeeping gene assay generates a

housekeeping assay result quantifying an amount of the housekeeping

messenger RNA present in the nucleic acid solution.

19. The method according to claim 18 further calculating a comparative

measure comparing the biomarker reverse transcription assay result to the

housekeeping gene reverse transcription assay result.

20. The method according to claim 19 wherein further comprising performing

steps a) to e) or more additional times on samples obtained at different time

points; and

      processing the comparative measures associated with the different

time points to infer a time-dependent host response.

21. The method according to claim 17 wherein the primer set is a first primer

set, and wherein the assay reagents further comprise a second primer set

configured to detect genomic DNA associated with a selected gene, wherein

the assay generates a biomarker reverse transcription assay result

quantifying an amount of the biomarker present in the nucleic acid solution

and a genomic DNA assay result quantifying an amount of the genomic DNA

present in the nucleic acid solution, and wherein signals respectively

associated with products amplified by the first primer set and the second

primer set are distinguished via melt curve analysis.

22. The method according to claim 21 wherein at least a portion of the

selected gene comprises at least a portion of an intron.

23. The method according to claim 21 further calculating a comparative measure comparing the biomarker reverse transcription assay result to the genomic DNA assay result.

24. The method according to claim 21 further comprising performing steps a) to e) or more additional times on samples obtained at different time points; and

  processing the comparative measures associated with the different time points to infer a time-dependent host response.

25. The method according to any one of claims 13 to 24 wherein steps b) to e) are performed in a disposable cartridge under the control of an automated instrument.

26. The method according to any one of claims 13 to 25 wherein the aqueous suspension is heated above at least 70 degrees Celsius.

27. The method according to any one of claims 13 to 25 wherein the aqueous suspension is heated above at least 80 degrees Celsius.

28. The method according to any one of claims 13 to 27 wherein the aqueous suspension is heated for at least one minute.

29. The method according to any one of claims 13 to 28 wherein the aqueous suspension is heated by Joule heating through an electric current applied thereto.

30. The method according to any one of claims 13 to 29 wherein the sample is a blood containing sample.

31. The method according to any one of claims 13 to 29 wherein the sample is a whole blood sample.

32. The method according to claim 31 wherein the whole blood sample is sampled directly into a blood collection vessel containing the lysis and stabilizing reagent.

33. The method according to claim 32 wherein the blood collection vessel is a PAXgene(TM) tube.

34. The method according to any one of claims 13 to 33 wherein the complex is separated centrifugally by:

centrifuging the mixture and precipitating the complex, thereby obtaining a precipitated complex;

centrifugally washing the precipitated complex one or more times.

35. The method according to any one of claims 13 to 33 wherein the complex is separated by filtration.

36. The method according to any one of claims 31 to 33 wherein the complex is separated centrifugally by:

centrifuging the mixture and precipitating the complex, thereby obtaining a precipitated complex;

centrifugally washing the precipitated complex two or more times.

FORMING A MIXTURE INCLUDING THE SAMPLE AND A LYSIS AND STABILIZING REAGENT, WHERE THE LYSIS AND STABILIZING REAGENT INCLUDES A CATIONIC DETERGENT CAPABLE OF LYSING CELLS PRESENT IN THE SAMPLE AND FORMING A COMPLEX WITH NUCLEIC ACIDS RELEASED FROM THE CELLS — 100

CENTRIFUGING THE MIXTURE AND PRECIPITATING THE COMPLEX — 105

CENTRIFUGALLY WASHING THE PRECIPITATED COMPLEX ONE OR MORE TIMES — 110

RESUSPENDING THE PRECIPITATED COMPLEX IN AN AQUEOUS SOLUTION — 115

HEATING THE AQUEOUS SUSPENSION TO A TEMPERATURE ABOVE 50 DEGREES FOR A TIME DURATION SUITABLE FOR DISSOCIATING THE COMPLEX AND RELEASING THE NUCLEIC ACIDS — 120

FIG. 1A

FORMING A MIXTURE INCLUDING THE SAMPLE AND A LYSIS AND STABILIZING REAGENT, WHERE THE LYSIS AND STABILIZING REAGENT INCLUDES A CATIONIC DETERGENT CAPABLE OF LYSING CELLS PRESENT IN THE SAMPLE AND FORMING A COMPLEX WITH NUCLEIC ACIDS RELEASED FROM THE CELLS — 100

CENTRIFUGING THE MIXTURE AND PRECIPITATING THE COMPLEX — 105

CENTRIFUGALLY WASHING THE PRECIPITATED COMPLEX ONE OR MORE TIMES — 110

RESUSPENDING THE PRECIPITATED COMPLEX IN AN AQUEOUS SOLUTION COMPATIBLE WITH DOWNSTREAM MOLECULAR ASSAY — 115A

HEATING THE AQUEOUS SUSPENSION TO A TEMPERATURE ABOVE 50 DEGREES FOR A TIME DURATION SUITABLE FOR DISSOCIATING THE COMPLEX AND RELEASING THE NUCLEIC ACIDS — 120

CONTACT NUCLEIC ACID SOLUTION WITH MOLECULAR AMPLIFICATION ASSAY REAGENTS — 125

PERFORM MOLECULAR AMPLIFICATION ASSAY — 130

FIG. 1B

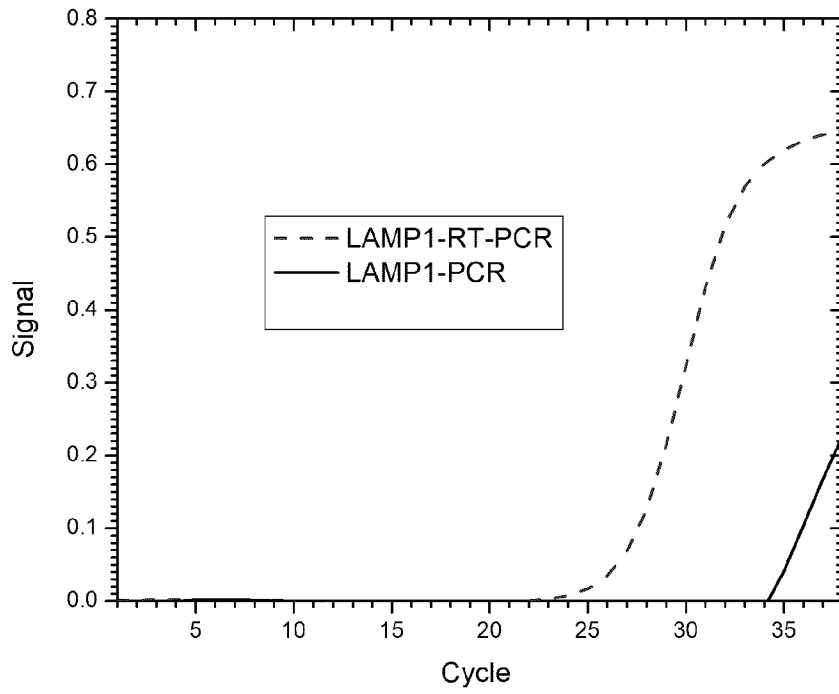| Storage time delay	2 hours
Storage temperature	Room temperature
First sedimentation volume	1mL mixture + 0 mL buffer
First sedimentation time	3 min
Number of washes	4
NAS-complex disintegration	Heat, $T_d=95$, $t_d=10$ min

FIG. 2A

FIG. 2B
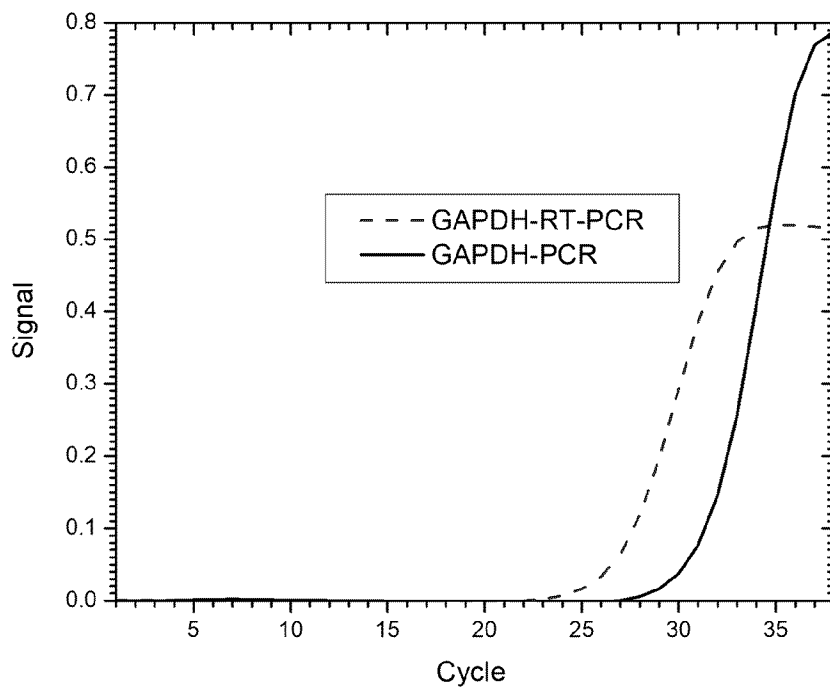
FIG. 2C
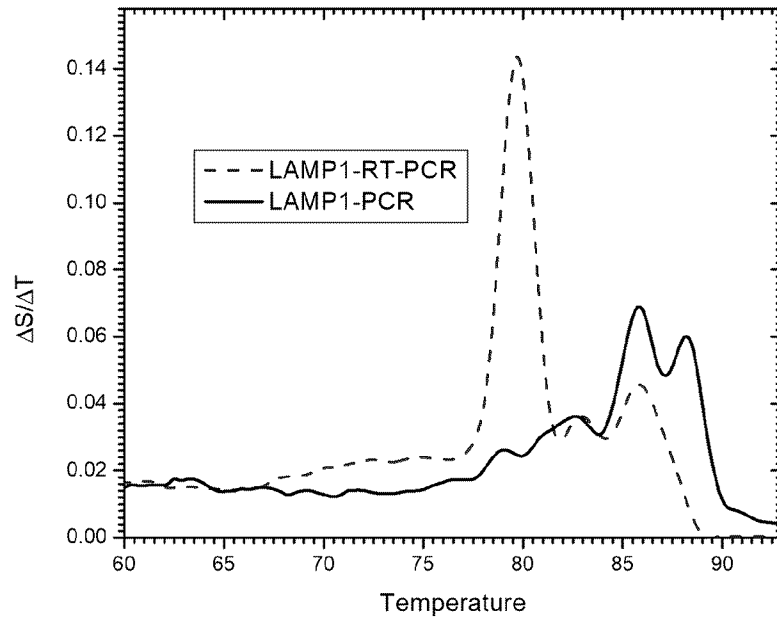
FIG. 2D
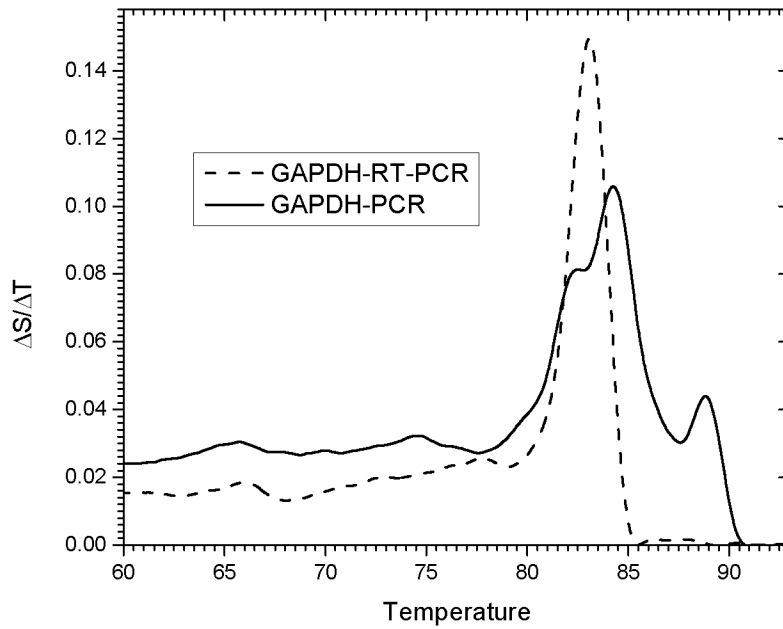
FIG. 2E

Storage time delay	**15 minutes to 3 days**
Storage temperature	room temperature or Freezing temperature
First sedimentation volume	1mL mixture + 9 mL buffer
First sedimentation time	10 min
Number of washes	3
NAS-complex disintegration	Heat, T_d=95, t_d=10 min

FIG. 3

Experiments	GAPDH		LAMP1	
	RT-PCR	PCR	RT-PCR	PCR
Storage time delay at room temperature				
15 min	27.73	31.28	29.17	33.33
1 hr	27.34	31.23	25.49	33.74
2 hr	28.08	31.53	25.48	33.50
3hr	28.07	31.55	27.88	33.56
1 Day	29.29	32.16	24.70	35.74
2 Days	29.76	33.30	29.47	36.42
3 Days	28.67	29.73	26.80	32.13
Frozen samples with storage time delay at room temperature				
1 hr	26.77	31.42	26.11	33.84
3 hr	28.66	32.27	26.86	33.99
5 hr	28.85	32.01	27.47	34.85
1 Day	29.56	32.25	26.63	33.21

FIG. 4

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Storage time delay	15 minutes to 2 hours at room temperature
Storage temperature	room temperature or frozen temperature
First sedimentation volume	1mL mixture + 0 mL buffer
First sedimentation time	1 to 10 min
Number of washes	4
NAS-complex disintegration	Heat, $T_d=95$, $t_d=10$ min

FIG. 5

Experiments	GAPDH		LAMP1	
	RT-PCR	PCR	RT-PCR	PCR
Storage time delay of 15 minutes at room temperature				
1 min	26.90	28.53	26.77	33.69
3 min	27.36	29.84	27.76	33.83
5 min	28.58	31.02	28.86	35.03
10 min	27.82	30.24	28.16	33.10
Storage time delay of 2 hours at room temperature				
1 min	27.46	30.54	27.57	34.59
3 min	27.02	31.04	27.05	36.29
5 min	26.19	29.80	27.19	36.05
10 min	27.53	29.06	27.97	35.31
Immediately frozen samples, storage time delay of 1 hour at room temperature				
1 min	26.35	29.74	25.71	37.93
3 min	26.78	30.82	25.78	N/D
5 min	27.87	30.10	26.76	N/D
10 min	27.52	30.43	26.60	N/D
Frozen after 3 hr at RT, storage time delay of 1 hour at room temperature				
1 min	27.03	29.44	25.53	N/D
3 min	27.39	29.47	26.69	37.76
5 min	27.23	29.56	26.04	N/D
10 min	27.31	29.39	26.96	N/D

FIG. 6

Storage time delay	1 hour at room temperature
Storage temperature	Frozen immediately
First sedimentation	1mL mixture + 0 mL buffer
First sedimentation time	3 min
Number of washes	4
NAS-complex disintegration	Heat, T_d=35 to 95, t_d=10 min

FIG. 7

Experiments	GAPDH		LAMP1	
	RT-PCR	PCR	RT-PCR	PCR
95°C	26.47	N/D	24.69	N/D
85°C	27.03	34.66	25.09	N/D
75°C	28.54	N/D	28.13	N/D
65°C	32.06	N/D	31.90	N/D
55°C	33.83	33.58	33.90	N/D
45°C	N/D	N/D	35.89	N/D
35°C	N/D	N/D	36.12	N/D
no treatment	N/D	N/D	N/D	N/D

FIG. 8A
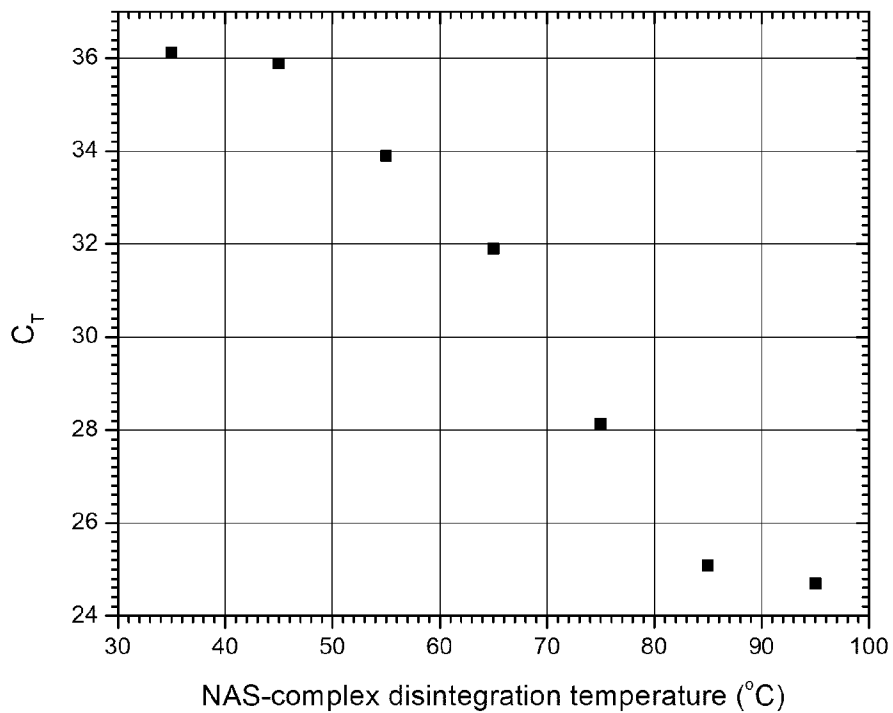
FIG. 8B

Storage time delay	1 hour at room temperature
Storage temperature	Frozen immediately
First sedimentation	1mL mixture + 0 mL buffer
First sedimentation time	3 min
Number of washes	4
NAS-complex disintegration	Heat, T_d= 95, t_d=1 to 10 min

FIG. 9

Experiments	GAPDH		LAMP1	
	RT-PCR	PCR	RT-PCR	PCR
1 min	27.19	33.33	26.33	35.88
3 min	25.74	30.20	25.49	34.20
5 min	25.71	30.74	25.46	33.37
10 min	26.47	28.17	25.69	31.15
no heat treatment	N/D	36.95	N/D	N/D

FIG. 10

Storage time delay	1 hour at room temperature
Storage temperature	Frozen immediately
First sedimentation	1mL mixture + 0 mL buffer
First sedimentation time	3 min
Number of washes	4
NAS-complex disintegration	Heat, T_d= 95, t_d= 10 min or Flash heating

FIG. 11

Experiments	GAPDH		LAMP1	
	RT-PCR	PCR	RT-PCR	PCR
Heat treatment-1	28.00	30.02	30.36	N/D
Flash heating -1	28.28	33.28	31.48	N/D
Heat treatment-2	27.27	29.99	28.61	N/D
Flash heating-2	26.43	28.49	29.02	33.85

FIG. 12

First sedimentation	1mL mixture + 1 mL buffer
First sedimentation time	3 min
Number of washes	1,2,3,4
NAS-complex disintegration	Heat, T_d= 95, t_d= 10 min

FIG. 13

Experiments	LAMP1	
	RT-PCR	**PCR**
1 wash	N/D	N/D
2 washes	32.78	N/D
3 washes	28.57	N/D
4 washes	28.07	37.73

FIG. 14

A. CLASSIFICATION OF SUBJECT MATTER

IPC: *C12Q 1/68* (2006.01), *C12Q 1/6806* (2018.01), *C12N 1/06* (2006.01), *C12N 15/10* (2006.01), *C12Q 1/6809* (2018.01), *C12Q 1/6844* (2018.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: ALL

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Databases: Questel Orbit, CIPO Library Discovery Tool, Google
Keywords: nucleic acid, stabiliz*, cationic detergent, surfactant, nucleic acid surfactant complex, distintegrat*, thermal processing, heating, heat treatment and transcriptomic profil*

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 2014/0199689 A1 (VOSS, T.) 17 July 2014 (17-07-2014)	1-4 and 6-12 5 and 13-36
Y	US 2016/0068897 A1 (TALEBPOUR, S. et al.) 10 March 2016 (10-03-2016)	5 and 13-36
A	US 7,683,035 B1 (ERBACHER, C. et al.) 23 March 2010 (23-03-2010)	
A	US 6,602,718 B1 (AUGELLO, FA. et al.) 05 August 2003 (05-08-2003)	
A	US 5,010,183 (MACFARLANE, DE. et al.) 23 April 1991 (23-04-1991)	

☐	Further documents are listed in the continuation of Box C.		☑	See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier application or patent but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search 23 January 2018 (22-01-2018)	Date of mailing of the international search report 01 February 2018 (01-02-2018)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Maria Mill (819) 639-6815

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
US2014199689A1	17 July 2014 (17-07-2014)	US2014199689A1	17 July 2014 (17-07-2014)
		US9695465B2	04 July 2017 (04-07-2017)
		DK2742152T3	31 July 2017 (31-07-2017)
		EP2742152A1	18 June 2014 (18-06-2014)
		EP2742152B1	12 April 2017 (12-04-2017)
		EP3216877A1	13 September 2017 (13-09-2017)
		JP2014525234A	29 September 2014 (29-09-2014)
		JP6096774B2	15 March 2017 (15-03-2017)
		WO2013024072A1	21 February 2013 (21-02-2013)
US2016068897A1	10 March 2016 (10-03-2016)	US2016068897A1	10 March 2016 (10-03-2016)
		US9707555B2	18 July 2017 (18-07-2017)
		CA2892813A1	05 June 2014 (05-06-2014)
		EP2925891A1	07 October 2015 (07-10-2015)
		EP2925891A4	10 August 2016 (10-08-2016)
		JP2016500008A	07 January 2016 (07-01-2016)
		US2014154687A1	05 June 2014 (05-06-2014)
		US8975060B2	10 March 2015 (10-03-2015)
		US2017275612A1	28 September 2017 (28-09-2017)
		WO2014082160A1	05 June 2014 (05-06-2014)
US7683035B1	23 March 2010 (23-03-2010)	US7683035B1	23 March 2010 (23-03-2010)
		AT496997T	15 February 2011 (15-02-2011)
		CA2299119A1	23 August 2000 (23-08-2000)
		CA2299119C	05 February 2013 (05-02-2013)
		DE50016065D1	10 March 2011 (10-03-2011)
		EP1031626A1	30 August 2000 (30-08-2000)
		EP1031626B1	26 January 2011 (26-01-2011)
		JP2000342259A	12 December 2000 (12-12-2000)
		JP4809967B2	09 November 2011 (09-11-2011)
US6602718B1	05 August 2003 (05-08-2003)	US6602718B1	05 August 2003 (05-08-2003)
		AT384954T	15 February 2008 (15-02-2008)
		CA2428864A1	18 July 2002 (18-07-2002)
		CA2428864C	12 April 2011 (12-04-2011)
		CN1503910A	09 June 2004 (09-06-2004)
		CN100386441C	07 May 2008 (07-05-2008)
		DE60132628D1	13 March 2008 (13-03-2008)
		DE60132628T2	29 January 2009 (29-01-2009)
		DK1356302T3	02 June 2008 (02-06-2008)
		EP1356302A2	29 October 2003 (29-10-2003)
		EP1356302B1	23 January 2008 (23-01-2008)
		HK1063503A1	06 February 2009 (06-02-2009)
		JP2004534731A	18 November 2004 (18-11-2004)
		JP5192631B2	08 May 2013 (08-05-2013)
		JP2010217198A	30 September 2010 (30-09-2010)
		US2002146677A1	10 October 2002 (10-10-2002)
		US6617170B2	09 September 2003 (09-09-2003)
		US2004048384A1	11 March 2004 (11-03-2004)
		US6821789B2	23 November 2004 (23-11-2004)
		US2004115689A1	17 June 2004 (17-06-2004)
		WO02056030A2	18 July 2002 (18-07-2002)
		WO02056030A3	28 August 2003 (28-08-2003)
US5010183A	23 April 1991 (23-04-1991)	None	